upon stimulation of the mental branch of the facial nerve. The results indicate involvement of the facial nucleus in this response.

Figure 2 shows examples of such recordings when the pressure on the nerve from the offending artery is eliminated and the response of the orbicularis oculi muscle vanishes suddenly; however, it often reappears, for example when a Teflon felt implant is put into place between the artery and the nerve. (These patients also show spontaneous contractions of the facial muscles during the operation which vanish after pressure is released, although not necessarily at the same time that the response to stimulation disappears.) We take this as an indication that the mechanisms behind this response are dependent upon the facial nerve being under pressure from the offending vessel. An example of a recording from the facial nerve when there is no response of the orbicularis oculi muscle upon stimulation of the distal end of the marginal mandibular nerve is seen in figure 2, B, together with a recording of the response of the orbicularis oculi muscle upon intracranial stimulation of the facial nerve near the REZ. This recording is shown with the stimulus occurring at the time of the first peak in the response from the facial nerve.

As may be seen from figure 2, B, the EMG response from the orbicularis oculi muscle in these two situations does not match in time as would have been the case if the response to stimulation of the distal part of the facial nerve was caused by ephaptic transmission in the facial nerve near the brainstem. A recording made a few seconds earlier than those seen in figure 2, B using the same stimulation of the marginal mandibular nerve elicited a second and later response from the facial nerve, the latency of which was 7.84 msec (fig. 2, D).

When the time of intracranial electrical stimulation of the facial nerve is aligned with the peak of the response from the facial nerve recorded when the mandibular nerve is stimulated, it becomes obvious that the response of the orbicularis oculi muscle coincides with the large and late response from that muscle obtained by stimulating the mandibular nerve. The latency of this response, together with the fact that this large response is labile in nature and it appears concomitantly with the response from the orbicularis oculi muscle, indicates that the response is generated in the facial nucleus and that it is directly related to the synkinesis seen when the mandibular nerve is electrically stimulated. It is thus assumed that it is this large response from the intracranial portion of the facial nerve that gives rise to the response from the orbicularis oculi muscle and not the smaller early response.

These results thus indicate that the facial motonucleus is involved in HFS. How this activity is generated in the motonucleus, however, is not obvious. It may be that the trigger zones developed in the injured part of the nerve give rise to unnatural neural activity that propagates in both directions from the trigger zone, and which is conducted antidromically by the facial nerve to the motonucleus. This bombardment of the facial motonucleus by such unnatural neural activity may give rise to changes in the motonucleus which are similar to 'kindling'[15,16]. After the facial motonucleus has been exposed to this unnatural stimulation, nerve activation such as electrical stimulation may trigger reverberant activity in the nucleus. This activity engages the entire nucleus, and thus all facial muscles are activated together (synkinesis). The same mechanism may be the cause of spontaneous spastic contraction of the facial muscles.

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Comparison of diurnal and nocturnal rates of 5-hydroxytryptamine turnover in the rat mediobasal hypothalamus[1]

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Summary. Rates of 5-hydroxytryptamine (5-HT) turnover in the mediobasal hypothalamus of male rats were estimated using pharmacological methods during the daytime and at night. Concentrations of 5-HT in this hypothalamic area were higher nocturnally than diurnally; this was apparently due to increased 5-HT synthesis and decreased 5-HT catabolism at night.
Key words. Rat hypothalamus; hypothalamus, rat; 5-hydroxytryptamine turnover, diurnal; 5-hydroxytryptamine turnover, nocturnal.

The hypothalamus contains relatively high concentrations of 5-hydroxytryptamine (5-HT; serotonin), and this indolamine plays a key role in the regulation of numerous hypothalamic functions, including that of modulating cyclic anterior pituitary secretions[2]. This modulatory role may be related, at least in part, to cyclicity in 5-HT metabolism within the hypothalamus. Although several other studies have reported apparent cyclicity in 5-HT concentrations in the hypothalamus of various species including rats[3-6], gerbils[7] and humans[8], changes in 5-HT concentrations may not accurately reflect neuronal activity associated with that neurotransmitter, as pointed out by Neff and Tozer[9]. Day-night differences in 5-HT metabolism estimated by the rate of formation of radioactively labeled 5-hydroxyindole acetic acid either from tritiated tryptophan[10] or

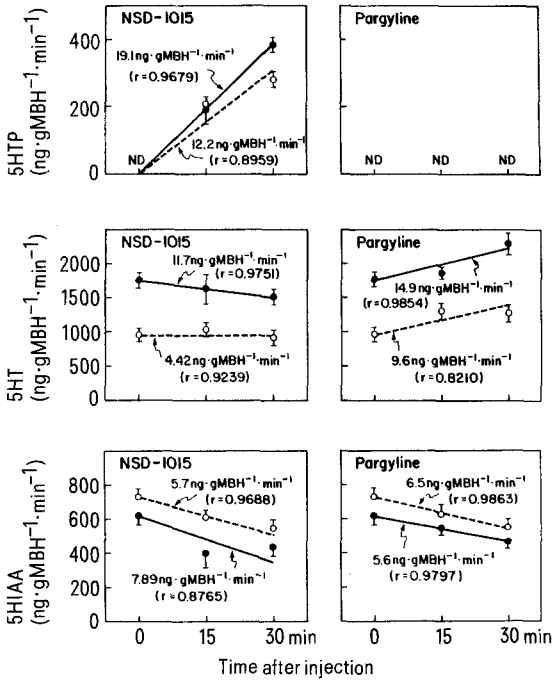
tritiated 5-HT[11] suggested an increase in 5-HT metabolism with the onset of darkness. However, isotopic methods used to determine 5-HT turnover rates are not without risk or complication[12]. As a result, most investigations involving 5-HT turnover employ pharmacological approaches. An appropriate choice of pharmacological approach deserves careful consideration, in view of the numerous hazards associated with these experimental tools. Therefore, the purpose of our study was to examine potential day-night differences both in 5-HT synthesis and catabolism in the mediobasal hypothalamus of the male rat comparing two commonly used pharmacological approaches to the study of 5-HT turnover; namely the use of pargyline, a monoamine oxidase inhibitor, or NSD-1015, an inhibitor of L-aromatic amino acid decarboxylase as well as monoamine oxidase.

Materials and methods. Forty-eight male Sprague-Dawley rats (120–140 g) were purchased from Simonsen Laboratories (Gilroy, CA). The rats were housed 4–5 per cage in an air-conditioned (22 ± 2 °C), windowless room. The automatically controlled light:dark cycle consisted of 12:12 LD (lights on at 06.00 h daily). The rats were provided with food (Wayne Lab-Blox) and fresh tap water ad libitum. Subgroups of eight rats were injected at 02.00 h (with the aid of a red-filtered [Wratten 1A, Kodak filter] 10 W light) or 14.00 h with (1) 200 mg·kg^{-1} of NSD-1015 (3-hydroxybenzylhydrazine; Sigma Chemical Co.) or (2) 75 mg·kg^{-1} of pargyline (N-methyl-N-2-propynylbenzylamine; Sigma Chemical Co.). Each drug was dissolved in 0.9% saline just prior to i.p. injection in a volume of 0.1 cm^3. The rats were subsequently killed by decapitation 15 or 30 min later. An additional subgroup of eight rats were injected i.p. with vehicle alone and killed immediately thereafter (either at 02.00 h or 14.00 h) to serve as controls. Within seconds after decapitation, the brain of each rat was removed and the mediobasal hypothalamus (MBH) was dissected as previously described[13]. Each MBH was then quickly frozen on dry ice and stored at −55 °C.

Within one day after sacrifice, the collected tissues were homogenized in 0.4 N perchloric acid containing 10^{-3} M sodium bisulfite. 3-Methoxy-5-hydroxyphenethyl alcohol (MOPET) was added to each sample and to the assay standard in order to estimate procedural losses. Aliquots of the tissue homogenates were centrifuged for 1 min at 12,000 × g. 5-Hydroxytryptophan, 5-HT and 5-HIAA were separated by high performance liquid chromatography (LCED) using a Biophase ODS, C-18 reverse phase column (25 cm × 4.5 mm; 5 µm particle size). The mobile phase consisted of 0.1 M sodium acetate, 0.1 M citric acid and 10% (v:v) methanol at a flow rate of 1.5 ml per min. An electrochemical detector with a carbon paste electrode maintained at a potential of 0.71 V was used to quantitate 5-HTP, 5-HT, 5-HIAA and MOPET as each eluted from the column. Unknown indolic peaks were identified by retention times compared to those of standards. Concentrations were determined by comparison of unknown indolic peak heights with those of standards. Values were corrected for the recovery of internal standard, averaging 98.2 ± 0.9%. Intra-assay variation was ± 4.1% for 5-HTP, ± 5.9% for 5-HT and ± 6.8% for 5-HIAA. Data are expressed as means ± SE of each mean. The initial indole concentration is considered as the concentration of that indole at time zero. The turnover rate (ng·g MBH^{-1}·min^{-1}) of indole efflux represents an estimate of the fraction of the indole pool (i.e., initial concentration) turned over or metabolized per MBH per unit of time. This estimate was calculated from the straight line plotted as the indole concentration versus time after drug injection by the method of least squares and is mathematically equivalent to the regression coefficient (or slope) for the line produced by this method. A two-tailed test for significant differences ($p < 0.05$) between regression coefficients (slopes) was employed to analyze differences between turnover rates[14].

Results and discussion. 5-Hydroxytryptamine concentrations were higher nocturnally than diurnally in the MBH of the male rat (1762.8 ± 51.3 ng·g MBH^{-1} vs 959.0 ± 45.1 ng·g MBH^{-1}, respectively; fig.). Various other studies have also reported higher 5-HT levels nocturnally in the hypothalamus of several species, including rats[3–6] gerbils[7] and humans[8]. Similar day-night differences in 5-HT concentrations have been observed in the rat suprachiasmatic nucleus and median eminence[10].

Brain levels of 5-HT have been demonstrated to increase linearly during the first 2 h following i.p. injection of pargyline (75 mg·kg^{-1})[9]. Due in part to the comparable results using this drug and other methods, pargyline has been a frequently-employed tool for estimation of 5-HT turnover rates. However, use of pargyline has several potential drawbacks. First, 5-HT may be metabolized by alternate pathways. For example, 5-HT could be O-sulfated. Gal reported formation and subsequent egression of 0.3 nmole·g^{-1}·h^{-1} of brain 5-HT-O-sulfate in rats treated with pargyline (80 mg·kg^{-1}, i.p.) and probenicid (200 mg·kg^{-1}, i.p.), a drug used to prevent 5-HIAA egression, suggesting a possible 20% underestimation of 5-HT turnover rates as a result of using pargyline[15]. Comparison of our estimated rates of 5-HT synthesis using 5-HTP accumulation after NSD-1015 treatment and 5-HT accumulation after pargyline treatment portrays a 21.9% lower estimated rate of 5-HT synthesis in the latter treatment group. An additional source of problems in the use of pargyline is the possible negative feedback influence which an accumulation of 5-HT might have on the rate of tryptophan hydroxylation[16]. Again, this could result in reduced 5-HT levels and an underestimation of the rate of 5-HT synthesis. Administration of NSD-1015 led to linear increases in 5-HTP levels. The rates of 5-HT synthesis estimated from the linear increases in 5-HTP levels were higher nocturnally (19.1 ng·g MBH^{-1}·min^{-1}) than diurnally (12.2 ng·g



Groups of eight rats were injected either diurnally (○—○) or nocturnally (●—●) with the monoamine oxidase inhibitor pargyline (75 mg·kg^{-1}, i.p.) or NSD-1015 (200 mg·kg^{-1}, i.p.) which inhibits both L-aromatic amino acid decarboxylase and monoamine oxidase activity. The rats were then killed 15 or 30 min after the initial injection time. One group of eight rats was injected with 0.9% saline vehicle (i.p.) and killed at time zero. Concentrations of 5-HTP, 5-HT and 5-HIAA in the mediobasal hypothalamus (MBH) were analyzed by LCED. Slopes were calculated by regression analysis.

$MBH^{-1} \cdot min^{-1}$). Peripheral injection of NSD-1015 in a dose range of 100–200 $mg \cdot kg^{-1}$ has also been shown to inhibit 5-HT oxidative-deamination as well as 5-HTP decarboxylation [17–19]. This results in a linear increase in 5-HTP and a linear decrease in 5-HIAA while 5-HT levels remain unchanged (i.e., 'steady-state levels'). Thus, an obvious advantage in using a drug like NSD-1015, which inhibits both 5-HT synthesis and catabolism, to estimate 5-HT turnover rates is the avoidance of possible feedback effects on the rate of 5-HT synthesis.

Our results also demonstrated decreased rates of 5-HT catabolism nocturnally. The rate of decline in 5-HIAA levels following pargyline administration is often used as a measure of 5-HT synthesis[20]. In our study, the estimated rates of 5-HT oxidative-deamination in NSD-1015 and pargyline treated rats were not significantly different either diurnally or nocturnally, suggesting that treatment with NSD-1015 inhibited 5-HT oxidative-deamination to a degree comparable with that due to treatment with pargyline. The discrepancies among regression coefficients obtained from the rise in 5-HTP levels and the decline in 5-HIAA levels after treatment with NSD-1015 could be explained by the existence of multiple pools of 5-HT with different turnover rates[21].

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Thiarubrine A, a bioactive constituent of *Aspilia* (Asteraceae) consumed by wild chimpanzees

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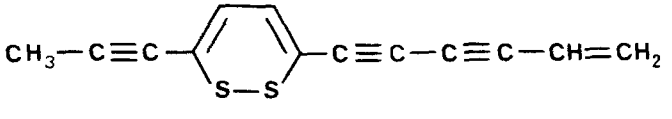
Summary. Two African species of *Aspilia* (Asteraceae), which are used medicinally by man and which are eaten by wild chimpanzees in an unusual manner, were found to contain the potent antibiotic thiarubrine A as a major leaf phytochemical. Its presence in leaf material strengthens the view that the feeding behavior of wild chimpanzees is related to special physiological or pharmacological effects on the animals.
Key words. Aspilia; chimpanzees, feeding behavior; thiarubrine A.

In various parts of Africa, numerous species of the genus *Aspilia* (Heliantheae, Asteraceae) are used for medicinal purposes[2]. For example, extracts of the root of *A. holstii* are prepared by the Shambala people for the relief of lumbago, sciatica and neuralgia[2]. The leaves of *Aspilia africana* from Nigeria, Cameroon and Ghana are used to clean sores, relieve stomach troubles, as a cough medicine, and in some cases, to remove corneal opacities[3]. More recently, Wrangham and Nishida[4] reported that wild chimpanzees *(Pan troglodytes)* selectively pick and swallow entire leaves of *A. mossambicensis* Oliv., *A. pluriseta* O. Hoffm., and *A. rudis* Oliv. without chewing them. This unusual feeding behavior by chimpanzees suggested to Wrangham and Nishida that the apes consume the leaves for some special pharmacological effects[4]. Janzen[5] has presented circumstantial evidence suggesting that certain mammals eat plants for their medicinal properties instead of for nutritional content. Ent-kaurenic diterpenoid acids have been reported from South American species of *Aspilia*[6] and an acetylenic compound has been identified in *A. montevidensis*[7]. There appear to be no phytochemical reports on African species of *Aspilia.*

Dried leaves of *Aspilia mossambicensis* from Mahale National Park and *A. pluriseta* from Kenya were finely ground and extracted with chloroform and taken to dryness in vacuo. The residue was dissolved in methanol and chromatographed in a Sephadex LH-20 column. Mass spectral analysis of a red oil



THIARUBRINE-A